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EDITORIAL

Genes in the Service of Therapeutic Index: Progress for Virus-Directed Enzyme Prodrug Therapy

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It has been a difficult past half-decade or so for would-be gene therapists. In contrast to the rather heady prospects in the early 1990s of using "gene surgery" to approach everything from congenital disease to cancer and HIV infection, recent reversals (including unforeseen toxicities encountered with adenovirus vectors and secondary lymphoproliferative disorders encountered with retroviral vectors) have tempered enthusiasm for these approaches in the clinical investigation community.[1,2] Despite these unfortunate outcomes, we cannot lose sight of the opportunities afforded by gene therapy approaches. Increased understanding of the biology of potential vectors for gene therapy, and our continuous advances in understanding the molecular basis for cancer will clearly lead to the design of new cancer treatments predicated on the expression of introduced genes into a tumor or its host.

Gene therapy can broadly be understood as promoting three types of outcomes. In the first, the gene(s) introduced into the host or tumor directly causes tumor cell death. The most frequently pursued paradigm is that of a replication-competent virus administered intralesionally. Replication of the virus in tumor cells, but not in normal host cells, leads to tumor cell death. Strategies employing this approach include the ONYX-015 adenovirus,[3] which could be considered prototypic for the field. This virus was engineered with a deletion in its *E1B* gene. Since the *E1B* protein product binds to normal p53 to allow virus replication, it was hypothesized that this might result in ONYX-015 replicating in and destroying only p53-deficient cells, a circumstance that is very common in human tumors. ONYX-015 has been safely administered in several intratumoral early phase trials.[4] We now understand that ONYX-015 can actually replicate in cells with wild-type p53. In addition, it has been hypothesized that other functional defects in the p53 pathway, particularly involving deletion of p14ARF, might allow its replication in those cases. Recent evidence, however, suggests that the basis for its selective replication is more complex.[5]

Other "oncolytic" virus strategies are also in development. These include Newcastle disease–related virus PV701, administered systemically and associated with some responses in early clinical trials; G207, a herpes virus already studied in a locally administered phase I trial in glioma; and a variety of vector systems in advanced preclinical or early clinical trials, including polio virus and other RNA virus–derived constructs.[6-9] For this type of application, the key requirement is that the virus naturally or by alteration of its regulatory features cannot replicate in the host, or its replication in normal host cells conveys no adverse organismic effect. A frequent basis for the success of such strategies is that tumor cells display altered regulation of growth-regulatory pathways that also are important in virus replication, not only of p53, as discussed above in the case of adenoviruses, but also the protein kinase R and interferon-responsive pathways in the case of RNA viruses.[9]

In a second application, the genes introduced into host or tumor cells are expressed, and the resulting protein evokes an immune response directed against the tumor. In this connection, genes can either be introduced as naked DNA, commonly as plasmids containing the gene of interest, or as viruses that are replication incompetent, such as adenoviruses or pox viruses, but still capable of infecting cells and expressing genes, including a transgene that encodes the immunogenic protein.[10,11] In this application, key variables in addition to assuring lack of gene replication potential in the host or transmission through the host germ line, are the level of target protein production, how the protein(s) of interest are presented to the host immune

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system, and schedules of immunization that would afford the greatest host response.

The third general strategy for gene therapy of cancer is in which the gene delivered conveys intrinsic toxicity to a tumor cell, or allows a subsequently administered drug to cause the infected tumor cell to die. This latter strategy is the goal of "gene-directed enzyme prodrug therapy" (GDEPT),[12] a major new step as illustrated by the report by Palmer et al[13] in this issue of the *Journal of Clinical Oncology*. In this "virus-directed enzyme prodrug therapy" (VDEPT) version of GDEPT, a virus that is replication-incompetent in normal or tumor cells is engineered to produce an enzyme not normally expressed in tumor or, ideally, in any human cell. The virus is locally administered into a tumor mass, where it is taken up by tumor and/or normal stromal cells, which expresses the target enzyme. The substrate specificity of the enzyme allows it to interact with a systemically administered prodrug, which by itself is nontoxic to human tissues, but which is metabolized by the expressed enzyme in the tumor environment to create a toxic metabolite.

Palmer et al are to be congratulated for a tightly managed, step-by-step, logical strategy to implement this concept.[13] They build directly on well-conducted preclinical experiments that demonstrate noteworthy antiproliferative activity of CB1954, the prodrug employed here, in cells expressing the activating enzyme *Escherechia coli*–derived nitroreductase, but not in cells that do not express the enzyme. CB1954's metabolite is expected to be short-lived, and reactive with tumor cell DNA close to where it is generated with the production of DNA adducts and other DNA damage. They have shown previously that CB1954 can be safely administered to humans and can achieve concentrations associated with activity in animal models bearing enzyme-expressing cells.[14] This article completes the other side of the "feasibility coin," demonstrating that the nitroreductase-expressing vector, CTL 102, when administered locally into tumors of patients who were candidates for resection of hepatic metastases, can express the reductase to levels that would be expected to allow clinical antitumor activity had the prodrug been administered. The investigators are now ideally positioned to move to the next clinical trial, in which patients with unresectable hepatic or other metastases will receive both the virus and, shortly thereafter, the prodrug.

Long-term observers of "tumor-targeting" strategies will recognize VDEPT as conceptually related to the previously pursued "antibody-dependent enzyme prodrug therapy" (ADEPT) strategy,[15] in which the metabolizing enzyme of interest was conjugated to a tumor cell–seeking antibody by chemical techniques before administration to tumor-bearing hosts. After allowing clearance of the antibody conjugate, a prodrug metabolized by the conjugated enzyme was given, analogous to the VDEPT strategy. VDEPT has many potential advantages in comparison to ADEPT. Specifically, cumbersome conjugation procedures linking antibody to enzyme can be avoided. Waiting for "washout" of non–tumor-bound antibody-enzyme conjugates is not an issue. Higher local levels of activating enzyme might be achieved by a virally expressed approach than might be achieved by antibody diffusing into tumor masses. The actual mass of virus required to get meaningful expression in tumors potentially requires less production space than the large amounts of protein required by antibody-enzyme conjugate approaches, so the cost of manufacturing is likely lower.

Other virus-delivered prodrug strategies have been described. The most frequently employed utilizes herpes virus–derived thymidine kinases (tks) to activate gancyclovir or a related drug to a toxic species. Early-phase trials of this general approach have been completed and, in some cases, are quite promising.[12,16] The strategy of Palmer et al,[13] however, is attractive for many reasons. If VDEPT approaches are to be of value, significant "bystander effect" killing must occur. That is, assuming even the best of vectors could not infect every tumor cell, the metabolite derived from the vector-expressed enzyme would then have to diffuse to other cells in the tumor. Because thymidine kinase–derived metabolites are charged phosphates, they require cell-to-cell junctional contact for this to occur. This is not expected to be the case with CB1954-derived metabolites. Second, antimetabolites require active incorporation into DNA, or act in some way related to nucleotide biosynthesis. This raises the possibility that vectors encoding their production would have the greatest value in tumors with high S phase fraction. In contrast, the CB1954-derived metabolite damages DNA independent of cell cycle phase, and thus represents a distinct approach.

Palmer's accomplishment is also notable for other reasons. First, they show clearly that expression of the transgene occurs despite some degree of pre-existing immunity to adenovirus, allaying the frequent concern that pre-existing antibody would attenuate the potential utility of such vectors. While it is true that titers of antiadenovirus and antinitroreductase antibodies increased during their study, it will be a matter of further clinical investigation in the event of promising initial results of the combination of virus plus prodrug to see how this outcome will affect the possibility of re-treatment. Second, as this was a series of patients in which operative removal of tumor was planned, the data obtained on expression of the transgene is compelling, and interestingly raises the possibility that some histologies (eg, hepatocellular carcinoma) may be better able to support transgene expression than others. Third, there is a close correspondence between expression of the Coxsackie adenovirus–receptor (CAR) and the nitroreductase, reenforcing the role of CAR in allowing uptake of the virus. Whether CAR expression should be a basis for stratifying

patients for entry into a study with these types of vectors logically emerges as a question from this outcome. Finally, the virus was administered directly into hepatic tumors with essentially no significant hepatic toxicity, and only transient exposure of the rest of the organism to detectable virus, at the higher doses and to a degree much lower than in the tumor cells.

Additional issues that were well addressed by this study relate to the care in choosing producer cell lines that do not allow significant generation of recombinant wild-type virus, and the definition of product characteristics that should allow facile manufacture in the event the approach actually is to be pursued. In pharmacologic terms, the importance of this study and of this gene therapy approach in general is that it may allow an outcome often missing in cancer therapeutics—a wide therapeutic index caused by introducing the key mediator of a chemotherapeutic agent's activation into the tumor environment. Analogous to the oft-quoted basis for success in the real estate market, pharmacologic "Location! Location! Location!" may now be redefinable in future approaches to locally problematic tumors by conveying "location-specific" toxicity under the influence of a virus-derived enzyme and a prodrug that is bland to the rest of the host.

Pitfalls might arise. For example, unexpected expression of the reductase in a site distant from injection could emerge; checking for transgene expression in other host tissues was not pursued in this study, quite reasonably, although the virus was only transiently detected in the circulation. Unexpected diffusion of the toxic metabolite out of the tumor could occur—a circumstance that should be limited by attention to dosing of vector and prodrug.

The oncology research and patient community awaits with great interest the next steps in this approach: the combination of the vector, CTL 102, and the prodrug, CB1954, in the successor to the clinical trial reported here. The gene therapy community also awaits eagerly this next step, as success here may give new impetus to a field that is definitely in need of good news.

Author's Disclosures of Potential Conflicts of Interest

The author indicated no potential conflicts of interest.

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